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Symposium on molecular biology of sexually transmitted diseases

This symposium, held on 23 March 1990 at the Royal Society of Medicine, London, organised by Dr Brian Evans and Dr David Wright of the Charing Cross Hospital, started with a review of reactive arthritis (RA), and in particular the sexually acquired reactive arthritis (SARA) following chlamydial infection. Dr Andrew Keat of the Westminster Hospital gave a brief review of the clinicopathological features, informing the audience that 1–3% of chlamydial infections resulted in aseptic arthritis; of these about 70% developed sacro-iliitis, 5–6% spondylitis and 20–30% enthesitis. The most investigated host determinant in the development of RA was the presence of the haplotype HLA-B27 which increased the risk of developing RA by 40%. There are seven HLA-B27 variants known but it has not been determined which of these variants predispose to RA. It is of note that there is extensive protein sequence homology between the HLA-B27 antigen and other MHC proteins, with the exception of the peptide binding region. Dr Keat's work centred on the hypothesis of antigen persistence. Specific cell-mediated and humoral responses to chlamydia remained enhanced in patients post-infection. Chlamydial antigen had also been demonstrated in synovial biopsies from SARA patients by immunofluorescence, and electron microscopy aided by immunoperoxidase staining. In a Westminster Hospital/Clinical Research Centre study of 133 rheumatology patients there were 9 SARA, 12 presumed SARA, 13 enteric reactive arthritis (ERA) and 3 both SARA and ERA. It was found possible to detect chlamydial antigen by immunofluorescence in some SARA patients but not in other groups. Genome detection by polymerase chain reaction (PCR) amplification of a 598 base pair sequence of invariate plasmid DNA was, however, negative in these antigen positive specimens.

Dr Ian Clarke of Southampton University discussed the molecular biology of *Chlamydia trachomatis*. There are 15 serovars of *C trachomatis* which are typed by sera reactive to a surface protein, the major outer membrane protein (MOMP). This is one of two major immunogenic proteins and is thus a target for potential vaccine development. The MOMP protein accounts for about 60% of the surface protein of the elementary body. It is a trimer of polypeptides interlinked by disulphide bonds. Functionally, it acts as a porin under redox control. The gene coding for the MOMP protein of *C trachomatis* serovar L1 has been cloned in *E coli* and subsequently sequenced. The predicted protein sequence is about 300 amino acids long, and has a high level of sequence homology with *C psitacci*. The conserved regions tend to be cysteine-rich. The variable regions appear to be immunodominant by epitope mapping. The other major immunogenic protein has a molecular weight of 60 kiloDaltons (kD), and is also cysteine-rich. Further work into vaccine development utilising either antigen is currently hampered by the absence of a suitable culture system for the generation of large amounts of antigen.

Dr Pamela Ewen from Cambridge reviewed the topic of allergic reactions to drugs. Most of the published work has been on penicillin hypersensitivity, the manifestations of which are well documented. Assessment of such reactions require: the documentation of the reaction; the timing, dose and route of administration of the drug; documentation of the disease being treated; a history of previous exposure to the drug; and noting of the effect of withdrawal of, or re-challenge with, the drug. The tests available to aid diagnosis, namely skin testing and measurement of specific IgE/IgG antibodies, are of comparable sensitivity and specificity. Skin testing with a major antigenic determinant of penicillin, benzyl penicilloyl polylysine, had been found to be safer than with benzylpenicillin. If alternative drug therapy was unavailable then a drug challenge could be tried. Specific regimes were outlined. Reactions to co-trimoxazole are of particular interest to STD physicians, in that rashes occurred in up to 50% of AIDS patients with *Pneumocystis carinii* pneumonia (PCP). As the incidence is lower in non-AIDS patients with PCP and the general population, it was postulated that the HIV virus played a role. Evidence to the contrary, however, was mentioned in discussion. Desensitisation regimes for both penicillin and co-trimoxazole have now been published. The difficult clinical problem of treating syphilis in pregnancy was also discussed at question time.

Professor David Mirelman of the Weizman Institute then talked about his work in a challenging talk entitled "Should all *Entamoeba histolytica* be considered potential pathogens?" He reviewed the spectrum of disease of amoebic disease, and asked whether there were two types of *E histolytica*, one pathogenic and the other non-pathogenic. There were biological differences between the two types in terms of ability to grow in axenic culture and isoenzyme profiles but it was not known whether these different genotypically, or merely expression of different phenotypes. In an elegant series of experiments where non-pathogenic organisms were grown in the presence of irradiated bacteria, it was possible to change the isoenzyme profile to that of a pathogenic zymodeme. It was also possible to show

by Southern hybridisation that tandem-repeat sequences thought characteristic of pathogenic types could, in fact, be detected in the non-pathogenic type but at very low copy number. If all *E histolytica* isolates have the capacity to be pathogenic, then is there an effective cure? Preliminary experiments were described by Professor Mirelman using silica molecules to which had been bound nitroimidazole. This compound was effective in vitro. The discussion provoked an alternative viewpoint from Dr Peter Sargeant, of the London School of Tropical Medicine and Hygiene, who felt that the clinical data available suggested that asymptomatic carriers rarely, if ever, became symptomatic.

After lunch, Dr Tim Harrison of the Royal Free Hospital School of Medicine gave a review of the molecular biology of hepatitis B virus. The pre-S1 and pre-S2 domains were now being incorporated into hepatitis B vaccines. The pre-S2 protein has been shown to promote attachment of the virus to hepatocytes and modulates the binding of polymerised human serum albumen to hepatitis B surface antigen. It is also involved in the control of virus assembly and maturation. Dr Harrison also summarised the retrovirus-like replication strategy of hepatitis B virus including the presence of reverse transcriptase activity and integration of viral DNA. The latter property is also a feature in hepatic carcinoma associated with hepatitis B infection. Some of the questions asked allowed the answering of some clinical points. Hepatitis core antibody alone does not confer protection. There were no specific markers of risk of infection. The pre-S2 containing vaccines were, probably, undergoing trials at the present time.

Professor Robin Weiss of the Institute for Cancer Research gave a masterly review of retrovirology. Retroviruses are not only a curse on mankind, they have given molecular biologists reverse transcriptase, oncogenes and expression vectors. They are now noted, however, for the diseases they cause. Apart from the well known diseases, they have also been associated with arthritis, osteopetrosis, encephalitis and pneumonia. These afflictions were not, of course, all in man but included diseases of such lowly creatures as the stony pike. The replication strategy of retroviruses was outlined, though it now appears that with the human immunodeficiency virus (HIV) integration of proviral DNA may not be a constant feature; it has been reported that HIV can grow in non-mitotically active cells. From the clinical viewpoint, of recent interest have been the diseases associated with the human T-cell leukaemia viruses (HTLV). HTLV-I has been shown to be aetiologically associated with adult T cell leukaemia and tropical spastic paraparesis (or HTLV-I associated myelopathy as it is known in Japan). In endemic areas the infection-case ratio of ATL is about 50:1. HTLV-II shares about 40% of its protein sequence with HTLV-I but is differentiable serologically. Areas of endemicity have not been found, though about 6% of intravenous drug abusers in London have been shown to have antibody to the virus. The virus has been causally related to the rare T cell variant of hairy cell leukaemia. The mechanism of cancer induction by the HTLV viruses seems to be different from other retroviruses, in that the viruses do not require integration by cellular oncogenes. They possess transactivating genes that up-regulate both viral and cellular genes. The prospects for new drugs and vaccines against retroviruses was briefly mentioned. Finally, Professor Weiss challenged the audience to research into the aetiology of Kaposi's sarcoma. The evidence suggests an infective aetiology.

Continuing the retrovirus theme, Dr Simon Wain-Hobson of the Pasteur Institute informed the meeting of the high degree of variability in gene sequence of HIV isolates, both in vitro and in vivo. In a four year period, HIV isolates from patients were examined at intervals by PCR amplification and sequencing; these isolates were both directly from peripheral blood lymphocytes and those passed in cell culture. There were sequence variants found in all the genes examined (tat, rev, nef/LTR). Moreover, the dominant "quasispecies" found in vivo were not necessarily selected as the dominant "quasispecies" in vitro. There were preferred base changes found, particularly guanosine to adenosine, and there were runs of nucleotide sequence changes interspersed with relatively conserved sequences. The presence of "quasispecies" needs to be taken into account when examining natural history studies.

The final talk was by Professor James Burnie of Manchester who spoke on his experience of typing clinical isolates of *Candida albicans* and cloning of the so-termed "47kD" antigen of the fungus. Candida species can be typed into serotypes, biotypes, morphotypes, by immunoblotting and by DNA fingerprinting for epidemiological and infection control purposes. The first four methods are crude and affected poor differentiation. DNA fingerprinting, by digestion of extracted DNA with the restriction endonuclease Eco R1, tended to show two dominant types. Examination of AIDS patients has not shown the presence of particular, associated DNA fingerprints. The 47 kD protein is thought to be a virulence factor. The gene has now been cloned and expressed in the gt11 vector, and by Western blotting with hyperimmune rabbit serum appears to be the breakdown product of a 92 kD precursor. The amino acid sequence of the parent molecule is approximately 85% homologous with the heat shock protein of another fungus, *Saccharomyces cerevisiae*; and also extensive homology with the other known heat shock protein sequences. Its role as a virulence factor is undergoing further investigation. Mannan has been thought of as another virulence factor, but this is now unclear.

The role of molecular biology in STD research is still in its childhood, but it is clear from this conference that it will play an ever more important rôle.

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